EVALUATING THE ANTIPROLIFERATIVE POTENTIAL OF METHONOLIC LEAF EXTRACT OF CASSIA NIGRICANS

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INTRODUCTION

Plants and plant based herbal preparations have been used to treat ailments since prehistoric times, and the treatment of various diseases with plant-based medicines has remained an integral part of many cultures across the globe. Side effects of several allopathic drugs and development of resistance to currently used drugs have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The World Health Organization estimates that 80% of the World’s population use herbal medicines in some aspects of primary healthcare and there is a growing tendency to “Go Natural” [1, 2]. It is well established that plants have always been a useful source, for occurrence of anticancer compounds [3-5]. Approximately 60% of currently used anticancer chemotherapeutic drugs (vinblastin, vincristine) are derived from plant resource [6, 7]. Although most of the plants used in the traditional medicine have been identified and their applications are well-documented, the anticancer efficacy of many plants is yet to be verified.

Cassia nigricans (Leguminosae family- Caesalpinioidae) is a woody annual herb or under shrub between 1.2 and 1.5 m high with small yellow flowers. It is widespread in India and tropical Africa including northern Nigeria, especially in cultivated in roadside and open grassy areas [8, 9]. They are well known in folk medicine for their laxative and for treating various skin diseases such as ring worm, scabies, eczema etc. [10, 11]. Further, they are known to be of high therapeutic value in ulcers, gastro-intestinal disorders, diarrhea [12]. The leaf extracts of C. nigricans have shown potent analgesic, anti-inflammatory, antimicrobial, larvicidal and anti-plasmodial activities [13, 14]. Although there is enough information on C. nigricans extracts use in various diseases’ treatment, however, literature survey revealed that there is paucity of data on its anticancer potential. Henceforth, in this study we have attempted to investigate the in vitro anti-cancer potential of methonolic extract of C. nigricans on breast cancer MCF-7 cell lines.

MATERIALS AND METHODS

Materials

All the chemical reagents and solvents of analytical grade were purchased from SRL Chemicals, India. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, No M5655) purchased from Sigma (St Louis, MO, USA).

Plant material and extracts preparation

The leaves of C. nigricans were collected from in the campus of Maharani Lakshmi Ammanni College For Women, Bangalore, India. The plant materials were authenticated by Dr. S. Sundara Rajan, a Taxonomist and the voucher specimen (MC-H-51) were deposited at the department. The leaves were cleaned with distilled water, dried and crushed into the fine powder by using an electric grinder. The coarsely powdered leaf material was extracted with pure methanol in a Soxhlet apparatus for 24 h. The extract was evaporated to dryness under reduced pressure using a Rotavapor (BuchiFlawil, Switzerland) and a portion of the residue was used for the anti-tumor test.

MTT assay

MCF-7 (breast cancer) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). All these cell lines were cultured and Cytotoxicity test were carried out using MTT assay [15,16]. The trypsized 70-80% confluent cell lines (MCF-7) of 1x10⁵ cells/well were seeded in a 96 well plate and incubate for 24 hr at 37 °C, and varying concentrations (0-500 µg/ml) of C. nigricans are added and incubated at 48 and 72 hrs. After incubation, 20 µL of MTT reagent will be added to each well and incubated for 4 hr at 37°C. The incubated cells were washed twice with PBS and DMSO (100 µl/well) reagent which dissolved the insoluble crystalline formazan product. The efficacy of the sample was determined based on the reduced dye at 570 nm by UV spectrophotometer. The effect
of the samples on the proliferation of MCF-7 cell lines were expressed as the % cell viability, using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100%.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation. The data in all the experiments were analyzed (Microsoft Excel 2007) for statistical significance using Students t-test and differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Plants have always been a potential source of new drug molecule and research work in this domain has resulted in discovery of more efficient drugs for cancer treatment [6, 7]. MTT is a simple, reliable technique, which measures cell viability and can be used for screening anti-proliferative agents [15, 16]. MTT assay is a spectrophotometric analysis, which uses [3-[4,5-dimethyl-thiazol-2-y]-2,5-diphenyl tetrazolium bromide], known as MTT, a yellow color and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slate purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and produced by the mitochondrial activity, is oxidized to MTT-formazan and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slate purplish color, which is insoluble in water. Then the oxidation of MTT is proportional to the mitochondrial activity and produced by the mitochondrial activity, is oxidized to MTT-formazan and water soluble compound. The MTT enters the cells through the plasma membrane and, in contact with superoxide produced by the mitochondrial activity, is oxidized to MTT-formazan, a slate purplish color, which is insoluble in water.

The 1,6,8- trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of Cassia nigricans (Leguminosae) seed shows larvicidal activity [11], and the anthraquinones that are present in the methanolic extract of Cassia nigricans [12] have led to the discovery of more effective drugs for cancer treatment.

The results of this study establish the anticancer activities of Cassia nigricans leaf extracts. The potential of Cassia nigricans extracts as anticancer activities may be due to the presence of phyto-constituents like anthraquinones. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of the plant as a source of easily available effective anticancer agents to the people, particularly in developing countries, including India.

Future work will be interesting to know the chemical composition and also better understand the mechanism of action present in the extract for developing it as drug for therapeutic application.

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Ethical Issues

There is none to be applied

CONFLICTS OF INTERESTS

Declared None

REFERENCES


"Drugs from Nature: Plants as an important source of pharmaceutically important metabolites" Guest Editor: Dr. Dhananjaya Bhadrupura Lakkappa